EPR ACTIVE BIOSCAVENGERS FOR ORGANOPHOSPHOROUS NERVE AGENTS

Research Thesis

Presented in partial fulfillment of the requirements for graduation with research distinction in Chemistry in the undergraduate colleges of The Ohio State University

by

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ABSTRACT

Organophosphorus containing nerve agents (OP's) are a threat to the general public due to their facile synthesis and high toxicity. The current methods for treatment require immediate intervention after exposure to the OP's. We are proposing the use of prophylactic bioscavenger butyrylcholinesterase (BuChE) as a preventative measure against these weapons. In order to better understand the biodistribution of these bioscavengers, we want to append a tag that will allow for in vivo measurement of the distribution. The tag we propose to use is a tetrathiatriarylmethyl radical. These compounds are stable in biological systems and can be observed by electron paramagnetic resonance (EPR) spectroscopy. Previously, these trityl radicals have been successfully prepared and used as sensors for molecular oxygen in biological systems. Enclosed are reports of improved large-scale synthesis of three different types of substituted aryl radical systems.

ACKNOWLEDGEMENTS

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF SCHEMES

CHAPTER 1

INTRODUCTION

Organophosphorus containing nerve agents (OP's) are a threat to the general public due to their facile synthesis and high toxicity. Their lethality presents itself by interrupting or stimulating the nervous system. As early as the 1930's, OP's were used as common insecticides and were later developed into neurotoxins. As synthesis of these nerve agents developed, numerous derivatives of OP's have been synthesized by the esterification of phosphoric acid.[1] Some structures of common OP's are shown in Figure 1.

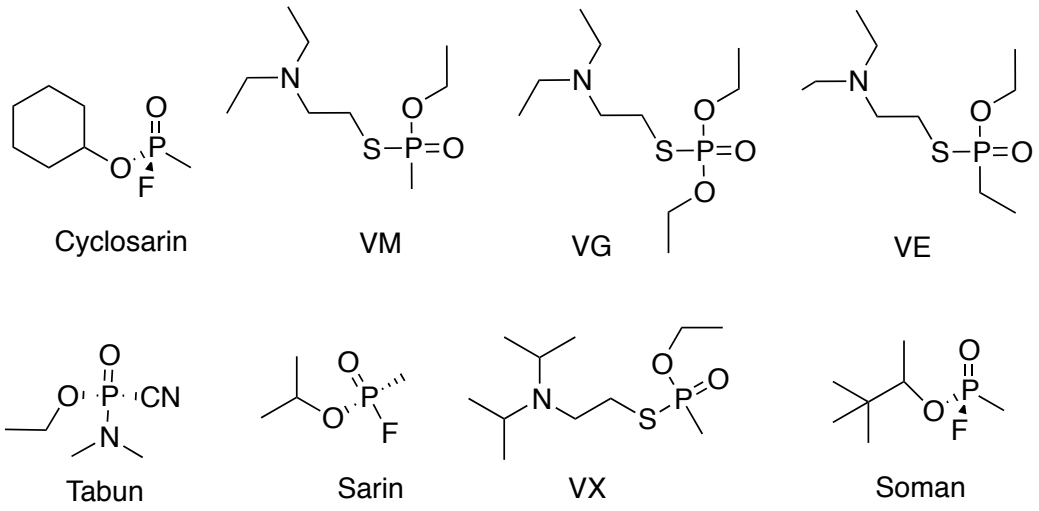

Figure 1: Structures of organophosphates.

Between 1961 and 1968, the United States synthesized approximately 4400 tons of VX for chemical warfare purposes. This amount had the potential to kill the entire population of the world. Today, organophosphorus compounds are a rising concern against the general public especially through the acts of terrorist groups and chemical warfare. OP's

have been used against civilian populations; and it is likely that they will be used again due to their ease of synthesis. "Approximately 3,000,000 people are exposed to organophosphates each year, with up to 300,000 fatalities. In the United States, there were more than 8,000 reported exposures to these agents in 2008, resulting in fewer than 15 deaths"[2,3,4] The ability to create chemical protection against these compounds is of great importance. [5,6]

OP's bind a serine residue (Ser-203) in the active site of AChE yielding a phosphorylated protein. Nucleophilic substitution by the hydroxyl group of Ser-203 yields the inhibited AChE. This first binding step is reversible with pharmaceuticals such as Pralidoxime, however, after a short amount of time ranging from minutes to hours depending on the size of the organophosphates R groups, binding becomes irreversible resulting in an aged AChE active site. This binding inhibits AChE indefinitely leading to the accumulation of acetylcholine in the nerve synapses.[8] This accumulation inevitably leads to the paralysis of neuromuscular function. Possible reversal of the phosphorylated protein involves the use of quinone methides (QM's) such as *o*-quinone methide. QM's are able to act as phosphoester alkylating agents. By alkylating the phosphate, there is potential for AChE to become reactivated, thus, eliminating any of the unfavorable effects caused by OP's. Specific pharmaceuticals for nerve agents include: Pralidoxime Chloride and Atropine shown in Figure 3.

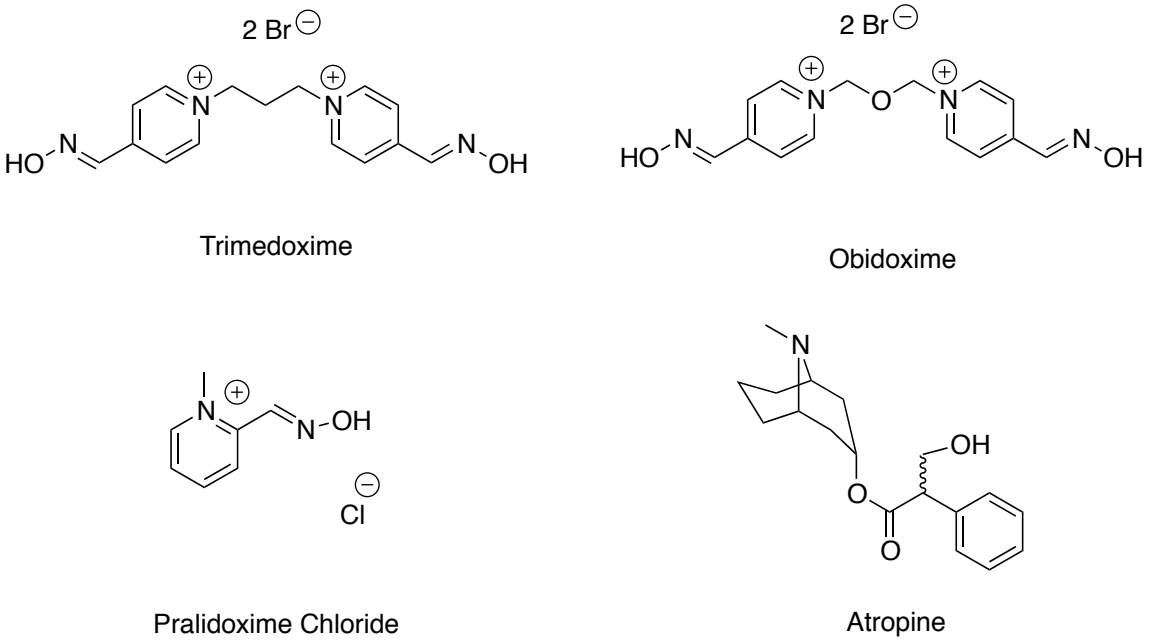

Figure 2: Structures of common oxime pharmaceuticals.

Pralidoxime chloride regenerates cholinesterase by reversing the phosphorylation of AChE previous to the aging process. Atropine has antagonistic affects at muscarinic receptors in the central nervous system.[8]

OP's are competitively bound or degraded by bioscavengers which affords a minimal level of protection to the critical enzyme acetylcholinesterase (AChE): "a key protein in control of neurotransmission at cholinergic synapses".[7] The inhibition of this enzyme affects "neuromuscular transmission" and in turn can cause loss of vital muscle functions by phosphorylating AChE.[7]

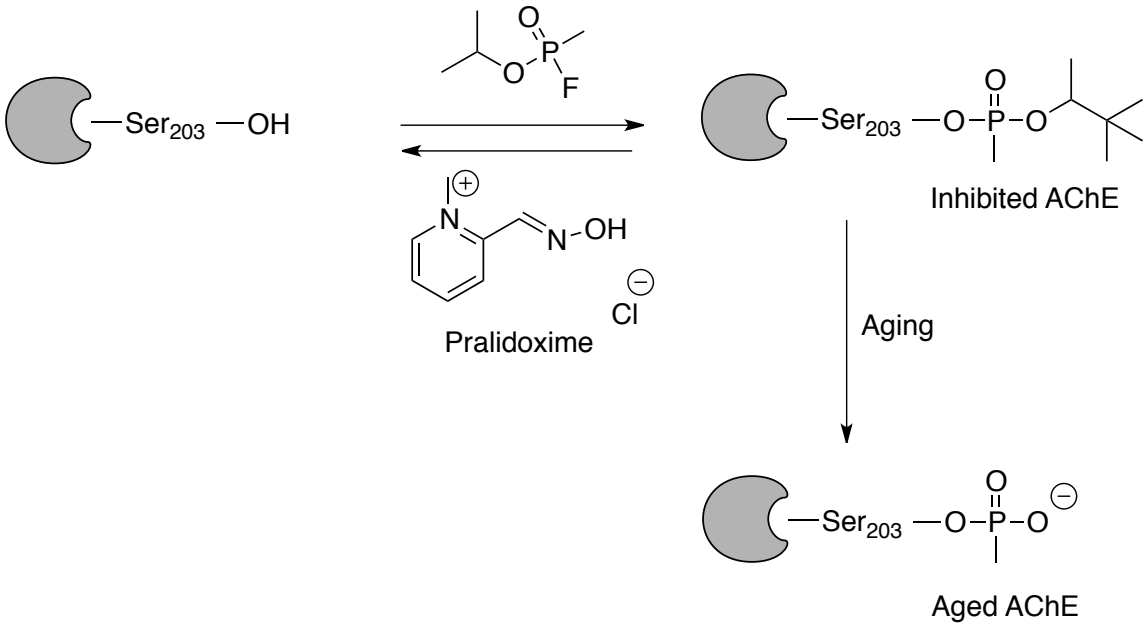

Figure 3: AChE phosphorylation and aging by OP's followed by dephosphorylation.

It is possible to use a prophylactic bioscavenger like butyrylcholinesterase (BuChE) as a preventative measure against these weapons. The ability to create some sort of protection against OP's is an advantage of bioscavengers: "protein-based enzymes of human origin capable of reacting with nerve agents and degrading them into harmless products." [8] OP's are competitively bound or degraded by these bioscavengers, which affords a minimum level of protection to the critical enzyme acetylcholinesterase. The ability of BuChE to catalyze the ester hydrolysis of acetylcholine to choline makes it similar to AChE.

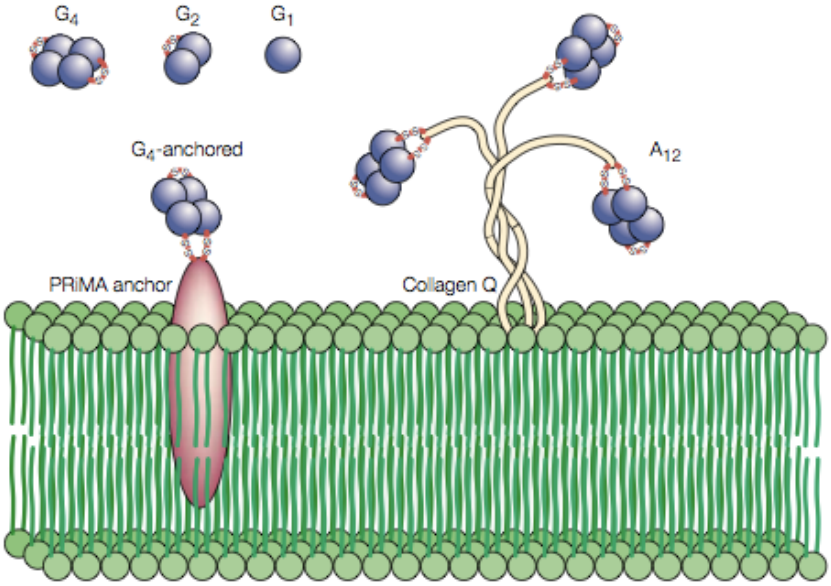

Figure 4: Various forms of BuChE and membrane interactions.[9]

In Figure 4, a schematic representation of the different molecular forms of BuChE and its ability to bind to membranes is shown. BuChE can exist in three soluble forms designated G_1 (symmetric monomeric), G_2 (dimeric), and G_4 (tetramer).[9] These forms of BuChE are typically bound to membranes through protein anchors that are rich in proline (PRiMA).[9] Other types of protein anchors include Collagen Q which has a triple helical form and allows for BuChE to assemble in the extracellular matrix.

Studies of BuChE's distribution in the brain have been extensively studied by using a variety of biochemical methods such as enzyme isolation in order to quantify the enzyme's concentrations. However, it is likely that the recorded concentrations were underestimated due to the reagents being used that inhibit BuChE.[9] Since the ability to monitor the biodistribution of biological agents is currently not well understood and is of great importance, adjusting the way to monitor BuChE *in* vivo requires specific

instrumentation. Understanding the biodistribution of protein-based therapeutics using electron paramagnetic resonance imaging (EPRI) is of great importance when determining molecular oxygen concentrations in the human body. This method has been proven to be effective for determining free radicals in biological systems. By appending the bioscavenger with a stable radical via a linker (Figure 5), its biological distribution could be measured *in vivo*.

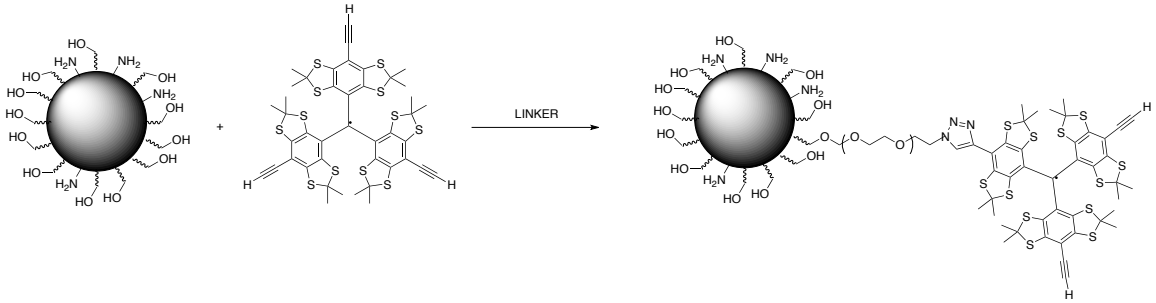

Figure 5: Trityl radical linkage to proteins via ether.

The goal is to develop a trityl radical library to use as biological sensors via EPR that will have multiple applications to study biodistribution. Applications include the testing of these EPR spin probes for conjugation to BuChE, treatment of enzymes inhibited by OP's in order to gain information of the bioscavenger, and applying this technology to other macromolecules to better understand their distribution *in vivo*.

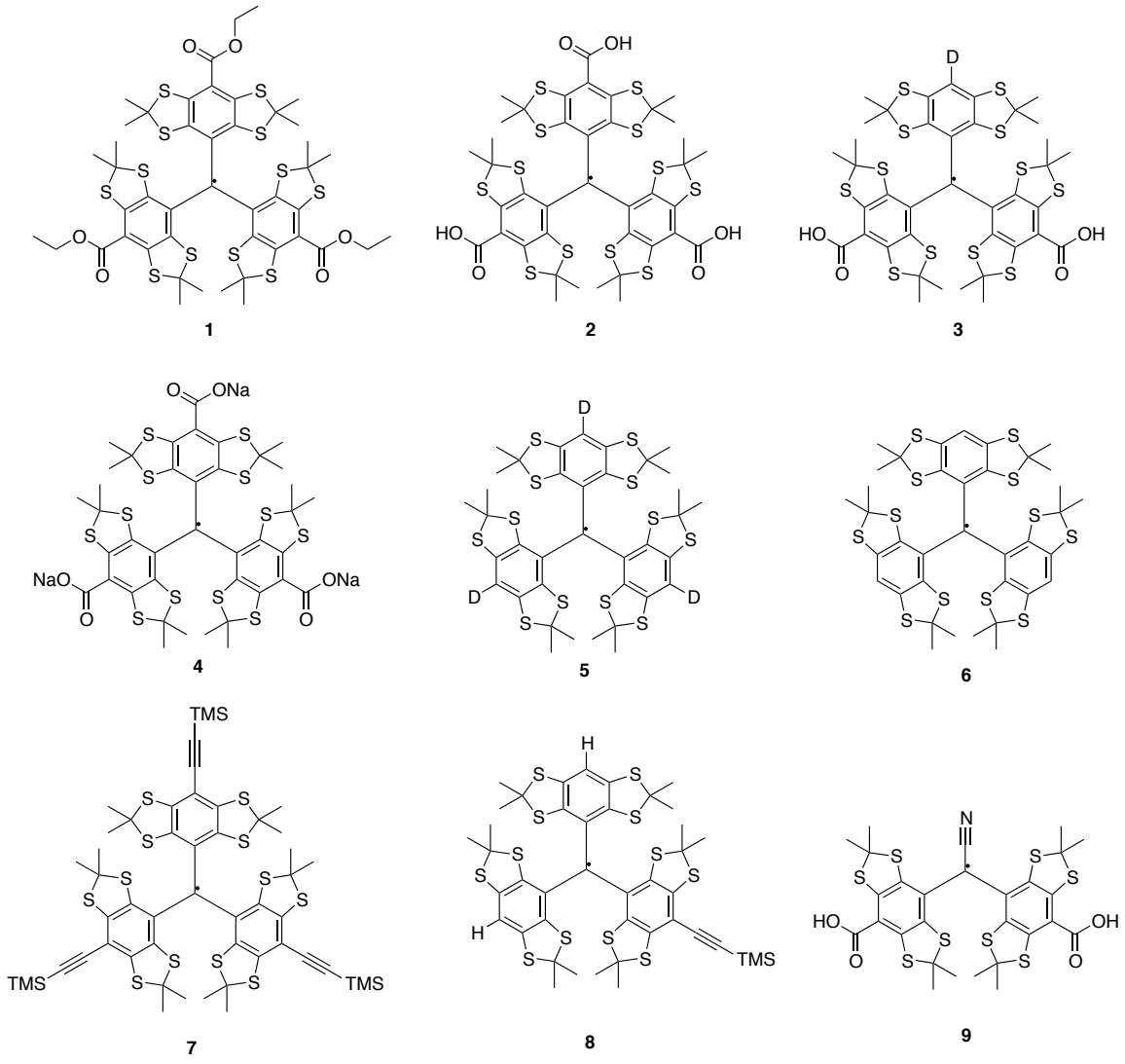

Figure 6: Trityl radical library of compounds.

Tetrathiatriarylmethyl radicals (Figure 6) are excellent probes in biological electron paramagnetic resonance spectroscopy. They demonstrate stability in biological tissue and have a half-life ranging from couple hours to 24 hours.[10] The development of these possible image-enhancing triarylmethal radicals will allow for effective spin labeling and a greater understanding of biological distribution *in-vivo*. The sulfur derivatives are of particular interest since they demonstrate greater advantages than those of the oxygen radicals. These advantages include an enhanced stability, improved water solubility, and high signal to noise. An example EPR is shown in Figure 7.[11]

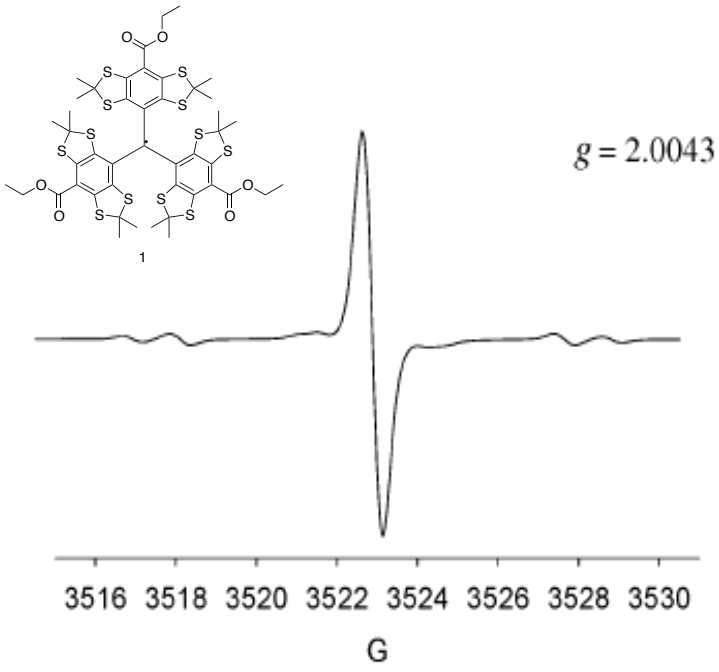

Figure 7: EPR spectra of trityl radical **1**.

In order for these compounds to be effective, it is important they are exemplified through a long half-life, do not induce an immune response, are water-soluble and demonstrate a

sharp signal in the EPRI. A sharp signal is necessary in order to obtain high sensitivity signals *in vivo*. Such a signal is achieved by eliminating any hyperfine couplings within the spectra. Signals acquired from such a spectrum is of importance because it aids in the understanding of the distribution of BuChE.[12, 13]

Currently many challenges exist when trying to determine a proteins biodistribution. A useful technique would be to tag a specific protein with a biomarker that would be able to be identified through non-invasive EPR spectroscopy. Advantages of non-invasive techniques such as EPRI compared to MRI for example is that "tagged proteins" can be determined at very low concentrations and this technique will allow for imaging of only the "tagged proteins". Hopefully through the continued experimentation and observations of the distribution of bioscavengers in a lab setting, understanding how trityl radicals degrade will probe improved stability for these radical systems. With this data in hand, we hope to perfect the synthesis of these biomarkers to the point of human use for a large amount of applications in understanding biodistribution.[12, 13]

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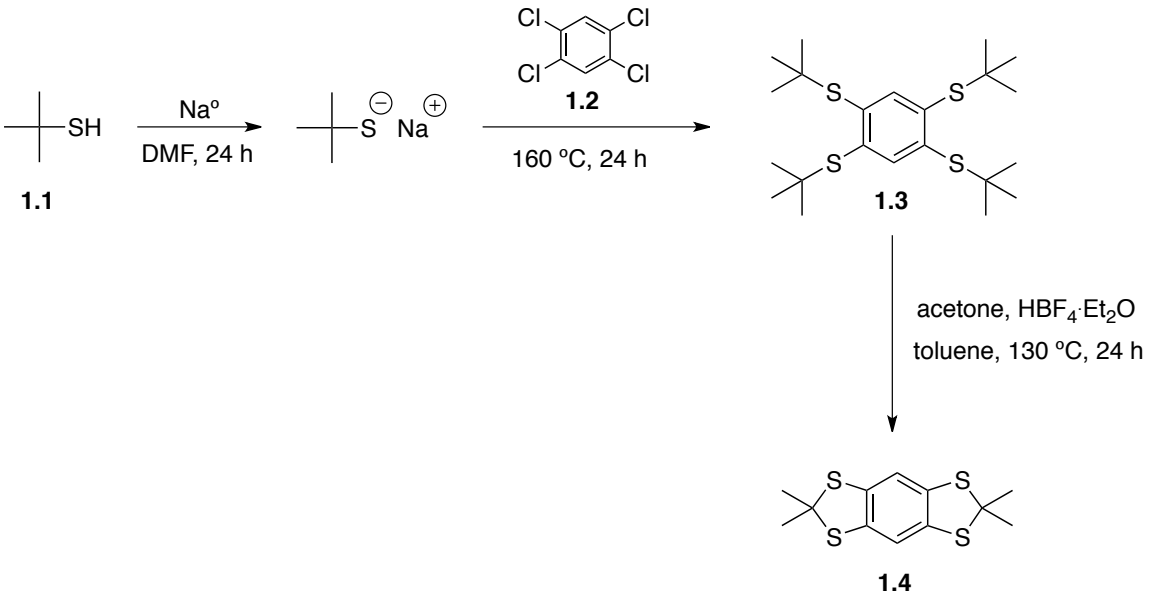
CHAPTER 2

SYNTHESIS OF ARYL TRITYL RADICALS

The formations of the initial precursors to all of the aryl trityl radicals in this thesis begin with 1,2,4,5-tetrachlorobenzene. These procedures have been optimized for the synthesis of all of the materials including the initial nucleophilic aromatic substitution reaction and thio-acetal formation shown in Scheme 2.1.[1, 2, 3, 4]



Scheme 2.1. Nucleophilic aromatic substitution and acetal formation **1.4**.

Compound **1.3** was formed by nucleophilic aromatic substitution on a suspension of sodium metal and **1.1** in DMF over a period of 4 h. The resulting solution was refluxed at 160 ºC for 24 h. The product was precipitated and purified by washing with water to yield **1.2** as a grey solid (86%). GC/MS and NMR analysis of this compound verified it to be analytically pure. Compound **1.2** was synthesized by an acid catalyzed ether cleavage followed by acetal formation. To a stirred suspension of **1.2** in toluene, acetone and

$HBF_4 \cdot Et_2O$ was added. The resulting mixture was allowed to reflux at 130 ºC for 24 h. The product was isolated after neutralization with saturated sodium bicarbonate, and triturated with a 1:1 mixture of acetone/methanol to yield **1.3** as an off white solid (81%). Both steps were optimized for time, concentration, and yield by monitoring with GC/MS. It should also be noted that the reaction scale could be performed on excess of 100 g with out any chromatography to afford **1.4**. 1,2,4,5-tetra-*tert*-butylthiobenzene (**1.2**) was produced in greater yields than that from the literature (63%) due to optimized equivalents and conditions. When previous reports in the literature were attempted, GC/MS showed a mixture of both di- and tri-substituted butylthiobenzene after the allocated time. Our modified conditions provide tetra-substitution in excellent yield as shown in figure 9 and 10. [1, 2, 3, 4]

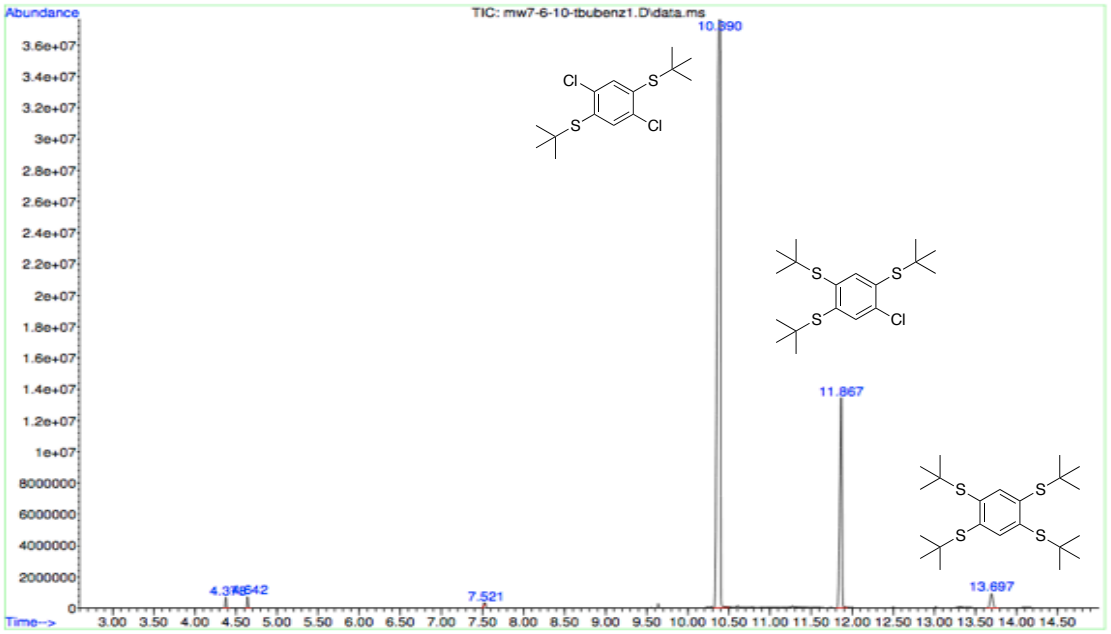


Figure 8: Gas chromatogram of compound **1.2** from literature procedure.

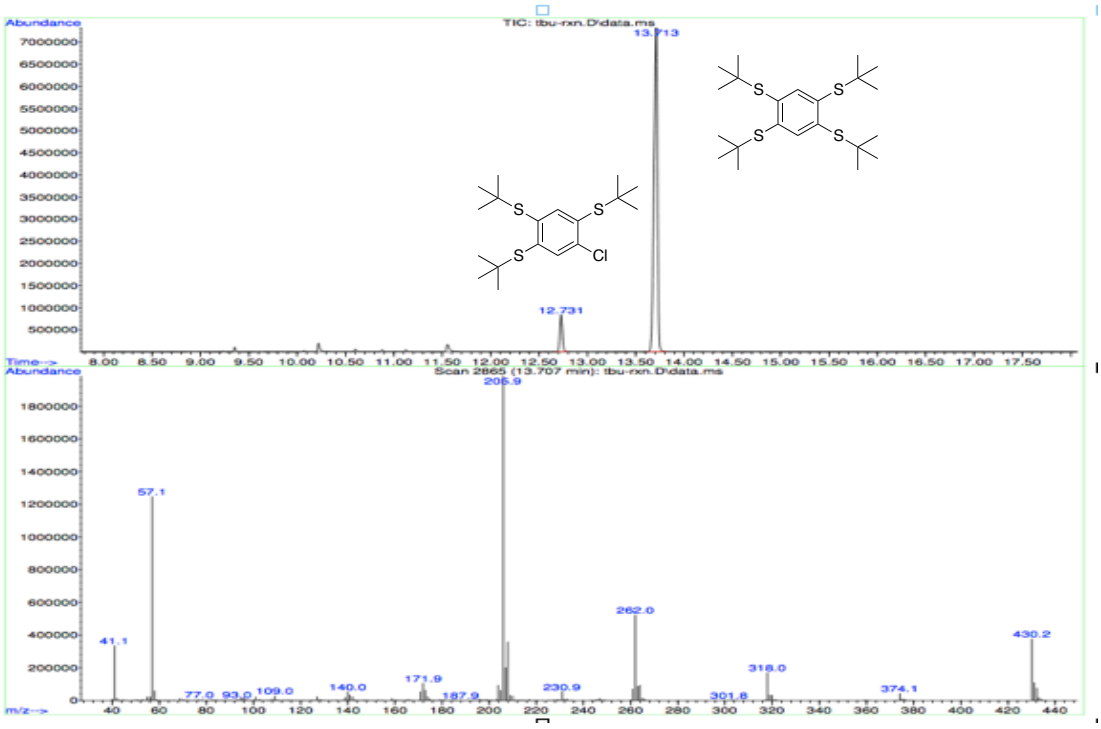
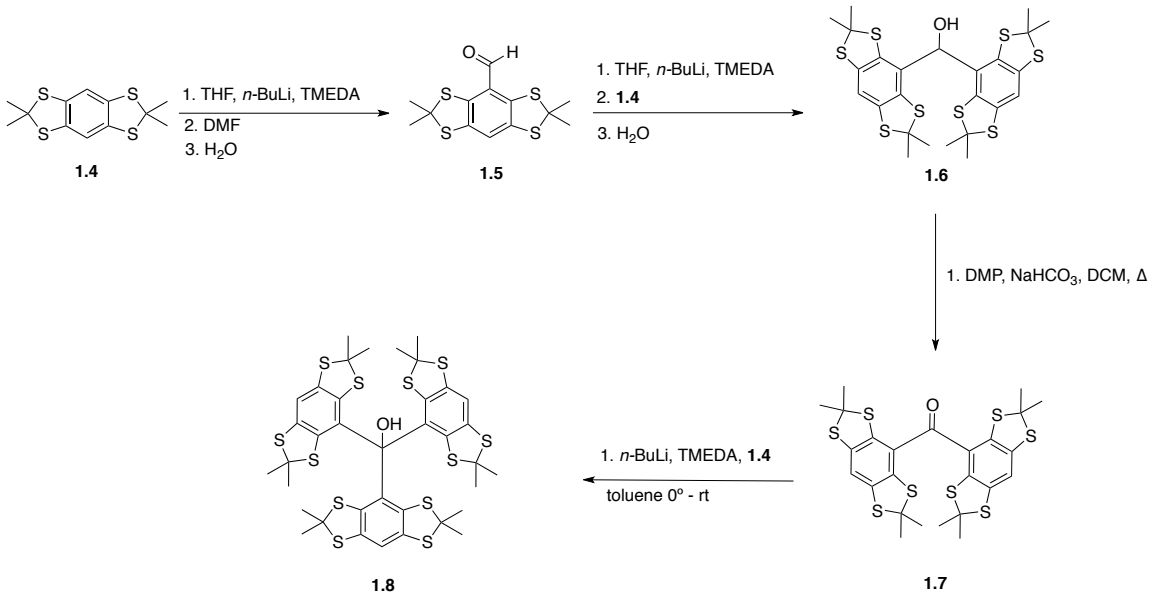


Figure 9: Gas chromatogram mass spectra of modified preparation of **1.2**.

The alternate synthesis optimized the equivalents, going from less than 5 to 6 equivalents of sodium and **1.1** and also increased the reaction time from 2 h to 24 h at reflux. Application of these alterations provided significant improvement of yield and purity.

With this starting material (**1.4**) in hand in large quantities we began our attempt to prepare the trityl radicals. A novel synthetic route was developed to produce the trityl alcohol (**1.8**) via formation of a ketone as shown in Scheme 2.2.

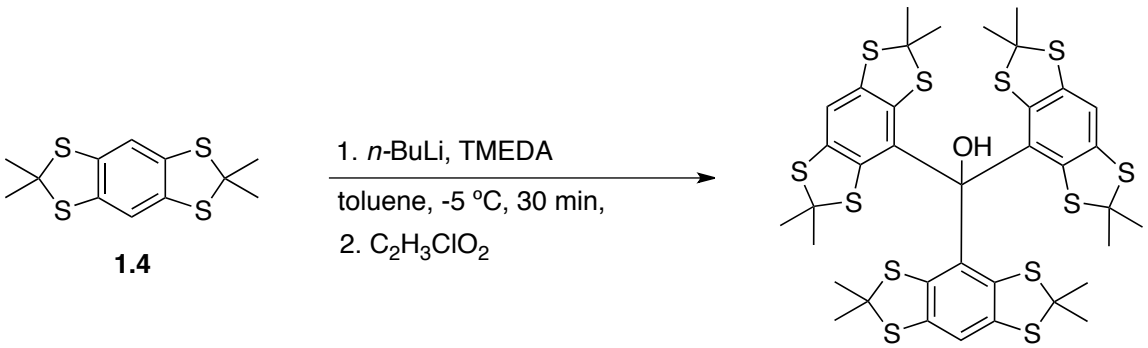


Scheme 2.2: Nucleophilic addition to Aldehyde and preparation of aryl anion **1.5**.

Compound **1.5** was synthesized via generation of the anion prepared from **1.4** with subsequent addition of DMF to yield the aldehyde. A suspension of **1.4** in THF, TMEDA and *n*-BuLi at 0 ºC were added respectively and the reaction was allowed to stir for 3 h, after which DMF was added. After purification by column chromatography a yield of 75% was achieved. Compound **1.6** was similarly synthesized by preparation of the bisthioacetal anion followed by the addition of the aldehyde to yield the benzyl alcohol

(**1.6**). To a suspension of **1.4** in THF, TMEDA was added followed by the addition of *n*-BuLi, dropwise at -78 ºC for 2 h. The resulting yellow oil was purified via column chromatography to give a yield of 65%. Compound **1.6** was then oxidized to **1.7** using Dess Martin Periodinane (DMP) and sodium bicarbonate. A slurry of **1.6**, DMP, sodium bicarbonate, and DCM was cooled to 0 ºC and monitored for complete oxidation by TLC. The slurry was extracted with DCM, evaporated down in vacuo and purified by column chromatography in a 98% yield. By generating the bisthioacetal anion followed by the addition of compound **1.7**, **1.8** was achieved in very low yields. We believe the yield to be low due to the bulkiness of the aryl monomers. Solvent concentration, temperature and time proved to be important. This alternate method to prepare **1.8** will allow for the propagation of unsymmetrical trityl alcohols that we will utilize in our future work.

We also optimized a method for synthesis of **1.8** by trimerization of **1.4** with methylchloroformate, TMEDA and *n*-BuLi to generate the trityl alcohol, **1.8** in a yield of 49%. [1, 2, 3, 4]



1.4 **1.8**

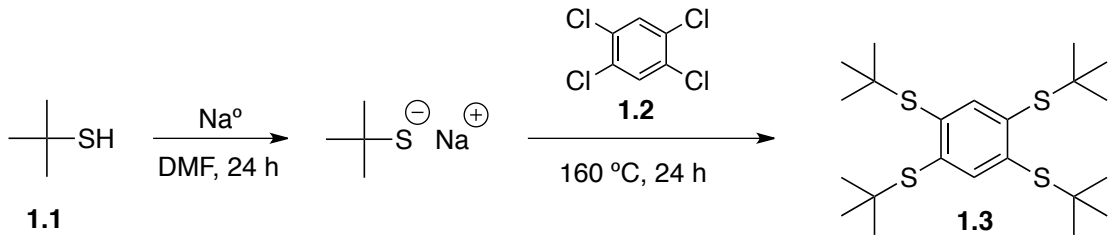
Scheme 2.3: Trimerization of anion with methyl chloroformate to yield **1.8**.

To a suspension of **1.4** and toluene, TMEDA was added, followed by the dropwise addition of *n*-BuLi at -5 ºC. The reaction was allowed to cool in an ice bath with calcium chloride for 30 min, then continued to stir for another 1.5 h. The reaction was allowed to stir for an additional 48 h and was purified using column chromatography to give **1.8**, a yellow powder in a 49% yield. The ability to trimerize **1.4** allows for optimized use of starting materials and a greater potential to make symmetric derivatives of the trityl alcohol by added various R groups to act as linkages for proteins. The ability to develop both synthetic routes allows for us to be able to utilize symmetric and asymmetric aryl trityl radicals.

Experimental

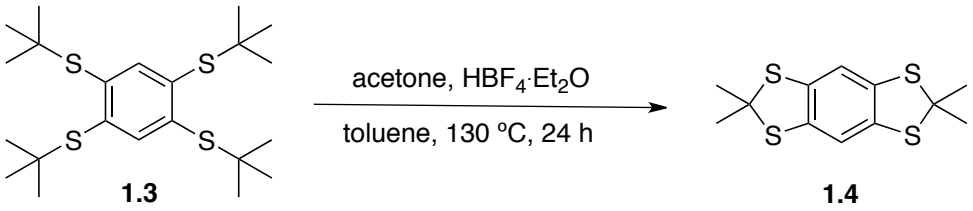
General. Solvents were distilled from the appropriate drying agents before use. Unless stated otherwise, all reactions were carried out under a positive pressure of nitrogen and were monitored by TLC on silica gel 60 F_{254} (0.25 mm, E. Merck). Spots were detected under UV light or by PMA in ethanol. Solvents were evaporated under reduced pressure and below 40 ºC (bath). Organic solutions of crude products were dried over anhydrous $MgSO_4$. Chromatography was performed on silica gel 60 (40-60 μM). The ration between silica gel and crude product ranged from 100 to 50:1 (w/w). Melting points are uncorrected. ^{1}H NMR spectra were recorded at 250 and 400 MHz, and chemical shifts are referenced to TMS (0.0, $CDCl_3$). ^{13}C NMR spectra were recorded at 100 MHz, and ^{13}C shifts are referenced to $CDCl_3$ (77.0, $CDCl_3$). Electrospray mass spectra were recorded on samples suspended in mixtures of THF and CH_3OH.

Experimental: Synthesis of **1.3**



1,2,4,5-Tetra-*tert*-butylthiobenzene (1.3). To a 1.0 L RBF equipped with a magnetic stirbar was added DMF (500 mL) and **1.1** (126 g, 158 mL, 1.392 mol). The mixture was stirred and small chunks of sodium were cut under hexanes and slowly added to the reaction over a 4 h period during which the color changed from yellow to dark red. After the sodium was completely dissolved, **1.2** (49.80 g, 0.2306 mol) was added and the mixture was refluxed in an oil bath at 160 ºC for 24 h. The mixture was cooled to rt (23 ºC) and was then poured over ice (500 g). The precipitate was isolated by vacuum filtration, washed with water (3 x 100 mL) to result in a grey solid (86.26 g, 0.200 mol, 86%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.36 (s, 36H), 7.74 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 31.1, 49.2, 134.4, 139.3; HRMS= 430.0886; R_f = 0.58

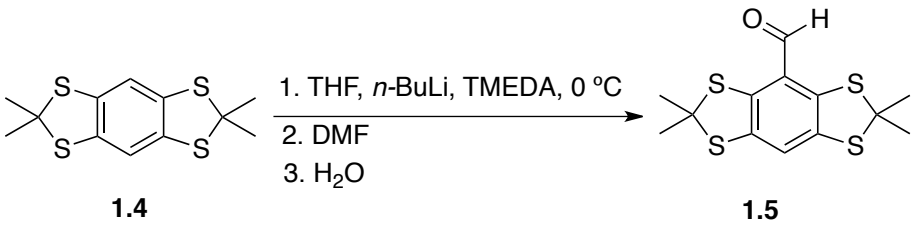
Experimental: Synthesis of **1.4**



1.3 **1.4**

2,2,6,6-Tetramethylbenzol[1,2-*d*;4,5-d]bis[1,3]dithiole (1.4). To a 1.0 L RBF equipped with a magnetic stirbar was added acetone (24 mL, 327 mmol), toluene (240 mL, 2.25 mol), and **1.3** (25.86 g, 60 mmol) at room temperature (23 ºC). $HBF_4{\cdot}Et_2O$ (12.36 mL) was added dropwise via syringe. The reaction was stirred for 4 h and then refluxed in an oil bath at 130 ºC for 24 h. The resulting black solution was allowed to cool to rt and extracted using diethyl ether (3 x 20 mL) and saturated sodium bicarbonate (2 x 120 mL). The combined organic phase was dried and evaporated under reduced pressure to a brown solid. The resulting solid was triturated with acetone/methanol (w:w) to yield a pale yellow solid (8.75 g, 30.60 mmol, 51%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.33 (s, 36H), 7.73 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 31.5, 49.2, 134.2, 140.0; HRMS=285.9946; R_f = 0.77

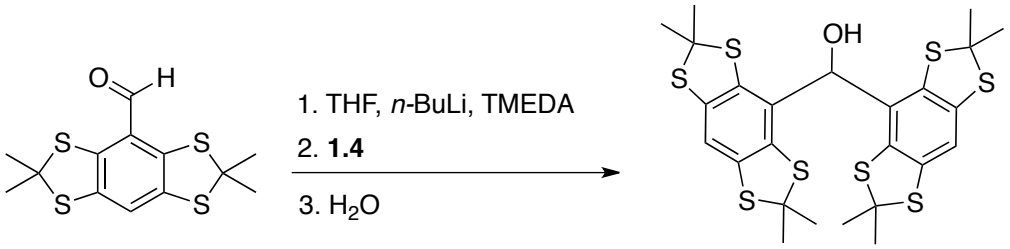
Experimental: Synthesis of **1.5**



1.4 **1.5**

2,2,6,6-tetramethylbenzo[1,2-*d*:4,5-*d'*]bis([1,3]dithiole)-4-carbaldehyde (1.5). To a flame dried 100 mL RBF containing **1.4** (1.80 g, 6.29 mmol) and magnetic stirbar under N_2, was charged with anhydrous THF (31.45 mL). The solution was cooled to 0 ºC and freshly distilled TMEDA (1.423 mL, 9.44 mmol) was added via syringe. *n*-BuLi (2.5 M in hexanes, 2.706 mL, 6.604 mmol) was added dropwise over a period of 5 min at 0 ºC. The ice bath was removed and the reaction stirred for 3 h. Anhydrous DMF (0.734 mL, 9.44 mmol) was added in one portion upon which the reaction went from dark red to a burnt orange in color. The reaction was allowed to stir for 24 h and water (4.0 mL) was added to the resulting yellow solution. The mixture was portioned with DCM and washed with 1M H_2SO_4 (2 x 20 mL) and saturated sodium bicarbonate. The combined organic phase was dried and the resulting orange liquid was evaporated under reduced pressure. The crude compound was purified by column chromatography (silica, 100:1 hexanes:EtOAc) to give **1.5** as a yellow-orange solid (1.481 g, 4.71 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.88 (s, 12H), 7.18 (s, 1H), 10.1 (s, 1H); ^{3}C NMR (100 MHz, $CDCl_3$) δ 31.7, 66.1, 120.9, 125.5, 137.9, 139.3, 188.8; HRMS=314.1072; R_f = 0.64

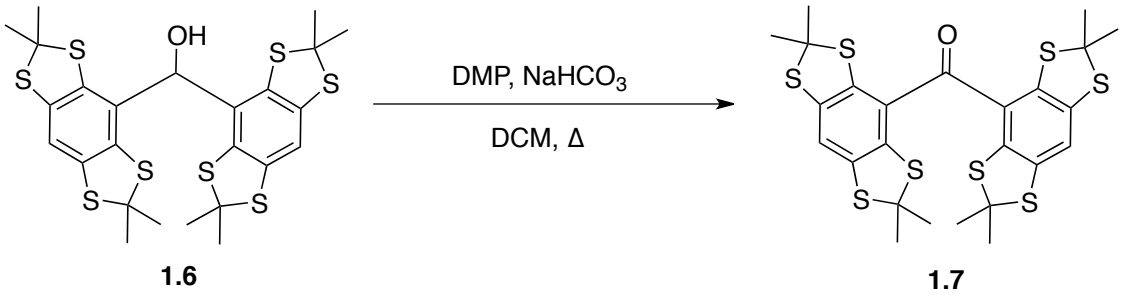
Experimental: Synthesis of **1.6**



1.5 **1.6**

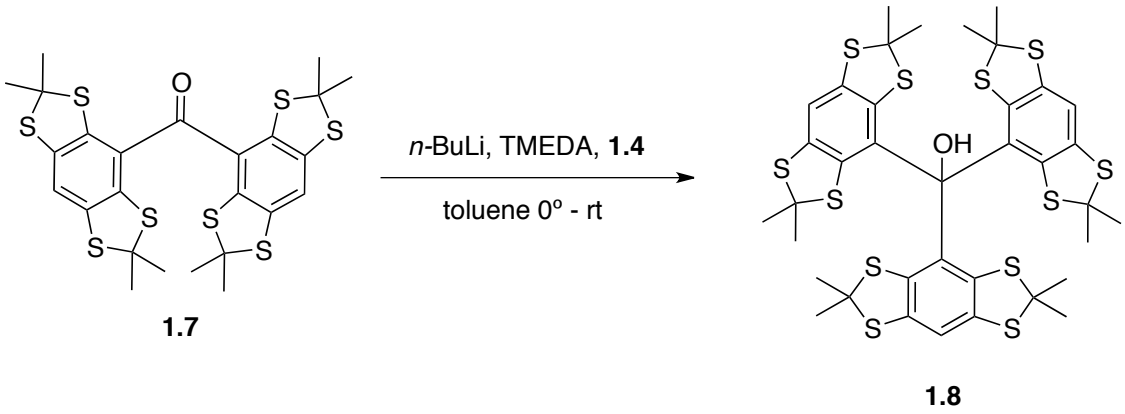
Bis(2,2,6,6-tetramethylbenzo[1,2-*d*:4,5-*d'*]bis([1,3]dithiole)-4-yl)methanol (1.6). A flame dried 50 mL RBF equipped with a magnetic stirbar and **1.4** (0.950 g, 3.32 mmol, 1.05 eq) was charged with anhydrous THF (16.6 mL) under an N_2 atmosphere at room temperature (23 ºC). Freshly distilled TMEDA (0.506 mL, 3.36 mmol, 1.06 eq) was added and the reaction was cooled to -78 ºC. *n*-BuLi (1.82M in hexanes, 1.85 mL, 1.06 eq) was added dropwise over 30 min while at -78 ºC. The reaction was kept at -78 ºC for 1 h. Compound **1.5** (1.0 g, 3.17 mmol, 1.0 eq) was dissolved in THF (10 mL) and added via cannula and the reaction was allowed to stir for 2 h at -78 ºC. The crude reaction mixture was portioned between water and DCM. The organic phase was washed with H_2SO_4 (1 M, 3 x 10 mL) and saturated $NaHCO_3$). The combined organic phase was dried, filtered, and concentrated to give crude brown-orange oil. The crude was purified by column chromatography (silica, 33% DCM in hexanes) which gave compound **1.6** as a yellow solid (1.16 g, 1.933 mmol, 61%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.82 (s, 24H), 2.64 (1, 2H), 5.67 (d, 1H), 7.06 (s, 1H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.6, 31.3, 116.0, 129.0, 135.4, 137.2; HRMS=599.9902; R_f = 0.59

Experimental: Synthesis of **1.7**



Bis(2,2,6,6-tetramethylbenzo[1,2-*d*:4,5-*d'*]bis([1,3]dithiole)-4-yl)methanone (1.7). To a 100 mL RBF equipped with magnetic stirbar was added **1.6** (1.074 g, 1.79 mmol, 1.0 eq), DMP (1.90 g, 4.48 mmol, 2.5 eq) and anhydrous sodium bicarbonate (0.15 g, 1.93 mmol, 1.0 eq). The mixture of solids was suspended in DCM (9.0 mL) while cooling to 0 ºC. After 10 min, the ice bath was removed and the reaction was monitored by TLC (75% EtOAc in hexanes). After completion, a bright orange color was observed. NaOH (10 mL). The mixture was then extracted with DCM (3 x 10 mL). The organic phase was dried, filtered and evaporated under reduced pressure to obtain a crude oil. The crude oil was purified by column chromatography (silica, 25% DCM in hexanes) to yield compound **1.7** as a bright orange solid (1.05 g, 1.75 mmol, 98%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.81 (s, 24H), 7.15 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 31.0, 65.2, 119.0, 127.0, 137.4, 193.2; HRMS=598.0231; R_f = 0.65

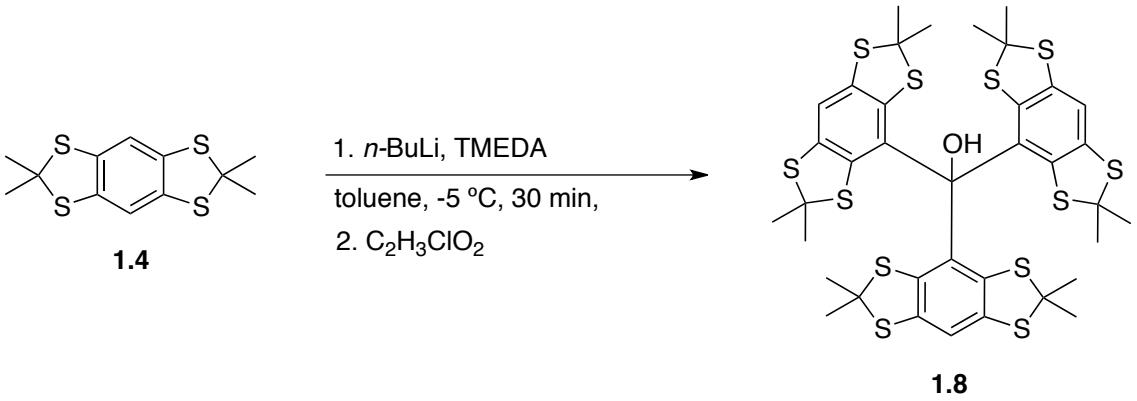
Experimental: Synthesis of **1.8**



1.7 **1.8**

Tris(2,2,6,6-tetramethylbenzo[1,2-*d*:4,5-*d'*]bis([1,3]dithiole)-4-yl)methanol (1.8). To a flame dried 100 mL RBF equipped with magnetic stirbar under N_2 was added **1.4** (0.40 g, 1.40 mmol, 2.1 eq) and toluene (10 mL). Distilled TMEDA (0.21 mL, 1.40 mmol, 2.10 eq) was added and the reaction was cooled to -78 ºC. *n*-BuLi (1.67 M in hexanes, 0.80 mL, 2.0 eq) was added dropwise over 5 min and the reaction was allowed to stir for 2 h. Compound **1.7** (0.402 g, 0.67 mmol, 1.0 eq) was dissolved in toluene (10 mL) and added via cannula, after which the ice bath was removed and the reaction was allowed to stir for 24 h. The reaction was extracted with DCM (3 x 10 mL), and washed with brine (20 mL). The organic phase was dried, filtered and evaporated under reduced pressure to yield a crude oil. The crude oil was purified with column chromatography (silica, 1% EtOAc in hexanes) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): ^{1H} NMR (400 MHz, CDCl3) δ 1.68 (s, 9H), 1.72 (s, 9H), 1.80 (s, 9H), 1.82 (s, 9H), 6.23 (s, 1H), 7.17 (s, 3H); HRMS= 885.0875; R_f = 0.74

Experimental: Trimerization of **1.4** to yield **1.8**



1.4 **1.8**

Tris(2,2,6,6-tetramethylbenzo[1,2-*d*:4,5-*d'*]bis([1,3]dithiole)-4-yl)methanol (1.8). To a flame dried 25 mL round bottomed flask equipped with magnetic stirbar, **1.4** (1 eq.) was added. The flask was purged with N_2 and charged with anhydrous toluene. Freshly distilled TMEDA (1 eq.) was then added to the reaction. The flask was cooled to -10 °C. To the mixture was added *n*-BuLi (1.1 eq) was added dropwise. Upon the addition of *n*-BuLi, the reaction changed from a pale transparent yellow to dark transparent orange. The reaction was kept at -10 °C for 30 min, at which point the ice bath was removed and the reaction was allowed to stir at room temperature (23 ºC) for 1.5 h. The reaction was cooled again to -10 °C and distilled methylchloroformate (0.33 eq) was added dropwise. The reaction was allowed to stir for an additional 18 h. The reaction was extracted with DCM (3 x 10 mL), and washed with brine (20 mL). The organic phase was dried, filtered and evaporated under reduced pressure to yield a crude oil. The crude oil was purified with column chromatography (silica, 1% EtOAc in hexanes) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.5 (s, 36H), 5.32 (s, 1H), 7.19 (s, 3H); HRMS=885.0575;

R_f = 0.74

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3. Xia, Shijing; Villamena, F.A.; Hadad, C. M. *J Org. Chem* **2006**, *71, 7268- 7279*.

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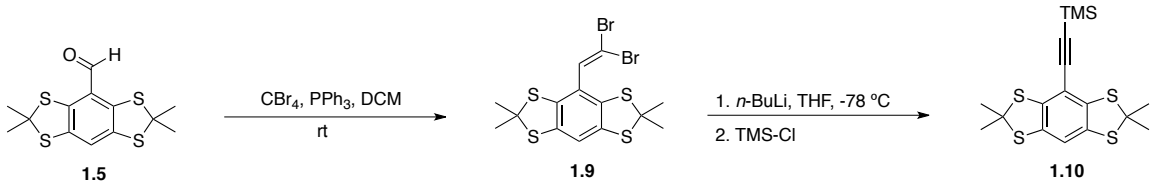
CHAPTER 3

STUDIES TOWARDS THE PREPARATION OF ARYL TRITYL RADICALS WITH LINKERS

Developing a new synthetic route that involves creating trityl radicals that can be used to link to proteins is the ultimate goal of this research. We hope that some of our previously described methods can be applied to synthesize compounds in good yields and high purity. These different linkages will allow for orthogonal protein tagging and different signals in EPRI. Formation of the TMS-alkyne begins with compound **1.5** and proceeds via a Corey Fuchs reaction in two steps to give compound **1.10**.

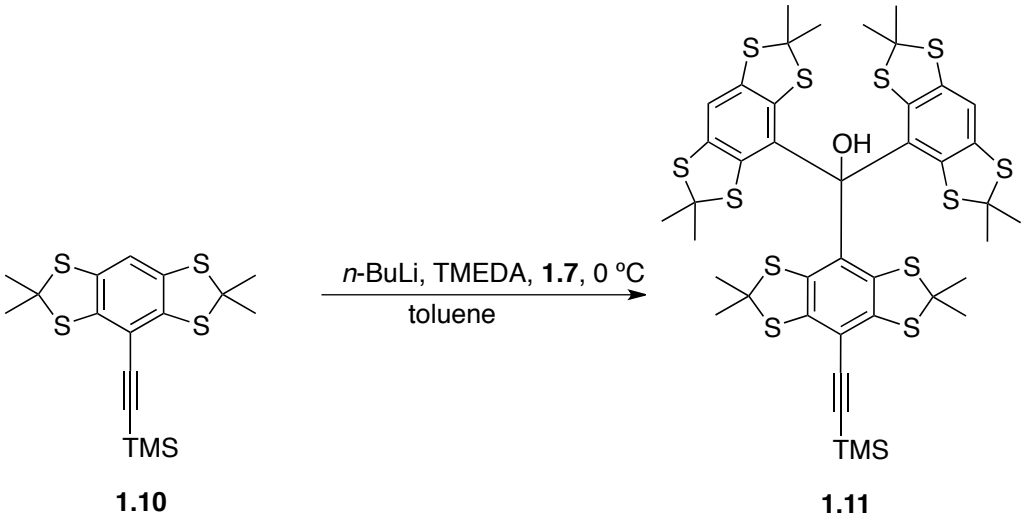


Scheme 3.1: Corey Fuchs reaction to prepare alkyne aryl precursor **1.10**.

Compound **1.9** was synthesized via a suspension of PPh_3 and CBr_4 in DCM followed by dropwise addition of compound **1.5** in DCM at -5 ºC. The reaction was allowed to stir for 1 h and monitored by TLC until completion. The product was isolated by column chromatography, in a yield of 86%. With **1.9** in hand, we converted it to the corresponding TMS-alkyne by adding it to a suspension of compound **1.9** in THF at – 78 ºC. *n*-BuLi was added over 5 min. The contents were allowed to stir for 3 h upon which

TMS-Cl was added. The reaction was allowed to stir for 15 h and then purified by column chromatography to yield compound **1.10** in a 64% yield.

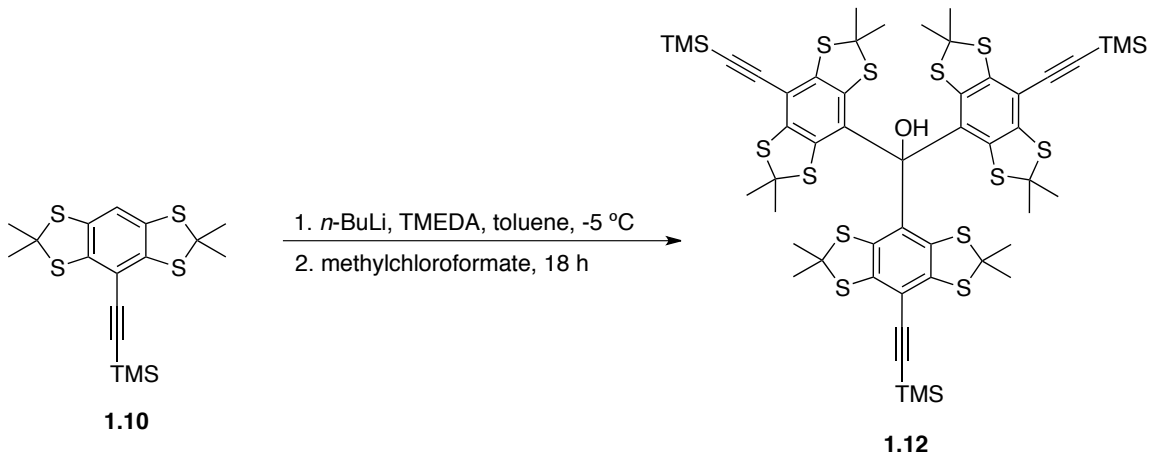
Compound **1.10** was then successfully converted to the tertiary alcohol **(1.11**) by addition to ketone **1.7** in low yield as previously observed.



Scheme 3.2: TMS-alkyne addition to produce trityl alcohol with TMS linker **1.11**.

To a suspension of compound **1.10** in toluene was added *n*-BuLi, TMEDA at 0 ºC. The reaction was allowed to stir for 2 h. Compound **1.7** was dissolved in toluene and added via cannula and monitored using TLC. The reaction was allowed to stir for 16 h and extracted in brine and DCM. The organic phase was purified using column chromatography to yield compound **1.11** in yield of 11%.

We also optimized a method for trimerizing **1.10** using TMEDA, *n*-BuLi, and methylchloroformate to generate the tri- substituted TMS trityl alcohol, **1.12**.



1.10 **1.12**

Scheme 3.3: Trimerization of anion with methyl chloroformate to yield **1.12**.

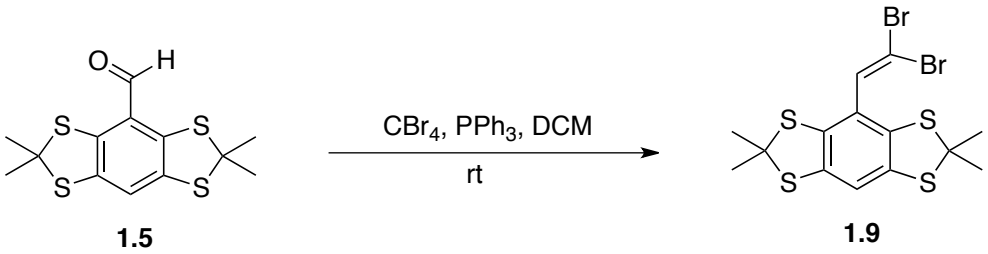
To a suspension of **1.10** and toluene, TMEDA was added, followed by the dropwise addition of *n*-BuLi at -5 ºC. The reaction was allowed to cool in an ice bath with calcium chloride for 30 min, then continued to stir for another 1.5 h. Methylchloroformate was added dropwise and allowed to come to rt over the next 18 h. The reaction was extracted with brine and DCM and purified using column chromatography to give **1.12**, a dark red oil in a 11% yield. The ability to trimerize **1.10** allows for optimized use of this new starting material and provides a greater potential to make symmetrical derivatives of the trityl alcohol by added various R groups other than TMS to act as linkages for proteins. It has been observed that the optimal synthesis of these trityl alcohols has been produce via the trimerization procedure. The initial synthetic route involved synthesis of the trityl

alcohol via Corey Fuchs followed by addition to compound **1.7**.

Experimental

General. Solvents were distilled from the appropriate drying agents before use. Unless stated otherwise, all reactions were carried out under a positive pressure of nitrogen and were monitored by TLC on silica gel 60 F_{254} (0.25 mm, E. Merck). Spots were detected under UV light or by PMA in ethanol. Solvents were evaporated under reduced pressure and below 40 ºC (bath). Organic solutions of crude products were dried over anhydrous $MgSO_4$. Chromatography was performed on silica gel 60 (40-60 μM). The ration between silica gel and crude product ranged from 100 to 50:1 (w/w). Melting points are uncorrected. ^1H NMR spectra were recorded at 250 and 400 MHz, and chemical shifts are referenced to TMS (0.0, $CDCl_3$). ^{13}C NMR spectra were recorded at 100 MHz, and ^{13}C shifts are referenced to $CDCl_3$ (77.0, $CDCl_3$). Electrospray mass spectra were recorded on samples suspended in mixtures of THF and CH_3OH.

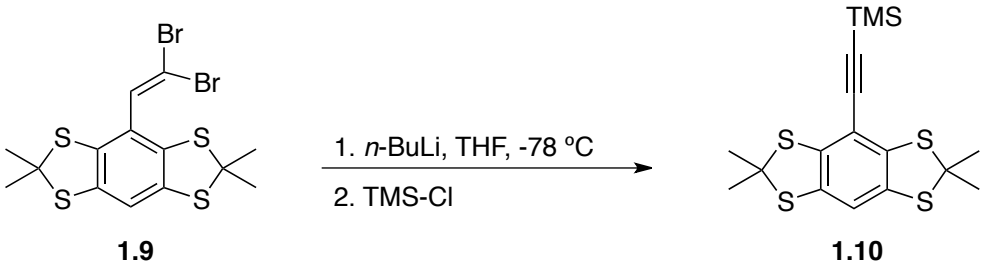
Experimental: Step 1 of Corey Fuchs reaction (**1.9**)



1.5 **1.9**

4-(2,2-dibromovinyl)-2,2,6,6-tetramethylbenzo[1,2-*d*:4,5-*d'*]bis([1,3]dithiole) (1.9). A flame dried 100 ml RBF equipped with magnetic stirbar was added CBr_4 (2.10 g, 6.36 mmol, 2.0 eq). The flask was purged with N_2, charged with anhydrous DCM (4.9 mL), and cooled to -5 ºC. PPh_3 (3.34 g, 12.72 mmol, 4.0 eq) was added via powder funnel and the reaction turned dark yellow/orange. The contents were allowed to stir for 30 min. Compound **1.5** (1.0 g, 3.18 mmol, 1 eq) was dissolved in DCM (40 mL) and added to the contents via cannula. TLC was used to monitor depletion of the starting material. DCM was added to the RBF and the crude organic phase was purified with column chromatography (silica, 0.33% EtOAc in hexanes) to yield **1.9** as a yellow solid (1.2732 g, 2,7215 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.91 (s, 12H), 7.03 (s, 1H), 7.30 (s, 1H); ^{13}C NMR (100 mHz, $CDCl_3$): δ 31.3, 65.5, 96.6, 116.4, 117.7, 126.1, 135.2, 135.3, 135.9; HRMS=467.8349 R_f= 0.61

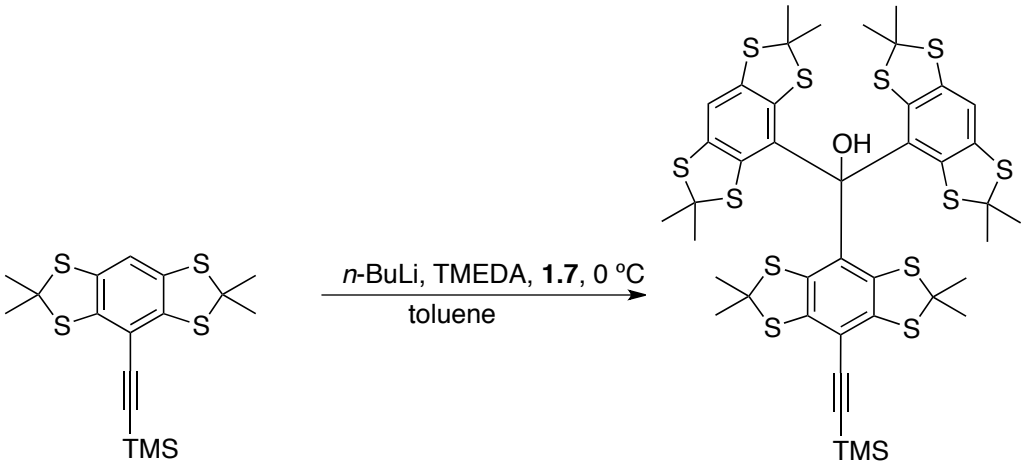
Experimental: Step 2 of Corey Fuchs reaction (**1.10**)



1.9 **1.10**

Trimethyl((2,2,6,6-tetramethylbenzo[1,2-*d*:4,5-*d'*]bis([1,3]dithiole)-4-yl)ethynyl)silane (1.10). A flame dried 100 mL RBF containing a magnetic stirbar, **1.9** (1.160 g, 2.479 mmol, 1.0 eq) was purged with N_2 and charged with anhydrous THF (25 mL). The reaction was cooled to -78 ºC and *n*-BuLi (2.5 M in hexanes, 2.28 mL, 2.3 eq) was added dropwise over 5 min. The reaction was allowed to stir for 3 h. TMS-Cl (0.808 g, 0.943 mL, 7.437 mmol, 3.0 eq) was added to the contents which turned a dark green color. The reaction was allowed to stir for an additional 16 h. Water (10 mL) was added and the reaction was extracted with DCM (3 x 10 mL), and washed with brine (20 mL). The organic phase was dried, filtered and evaporated under reduced pressure to yield a crude oil. The crude oil was purified with column chromatography (silica, 10% DCM in hexanes) to yield **1.10** as a yellow solid (0.6053 g, 1.593 mmol, 64 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.28 (s, 9H), 1.91 (d, 12H), 6.92 (s, 1H); ^{13}C NMR (100 mHz, $CDCl_3$): δ -0.05, 13.7, 22.0, 31.8, 64.7, 100.9, 104.6, 111.9, 115.9, 134.7, 139.5; HRMS= 382.0354; R_f = 0.61

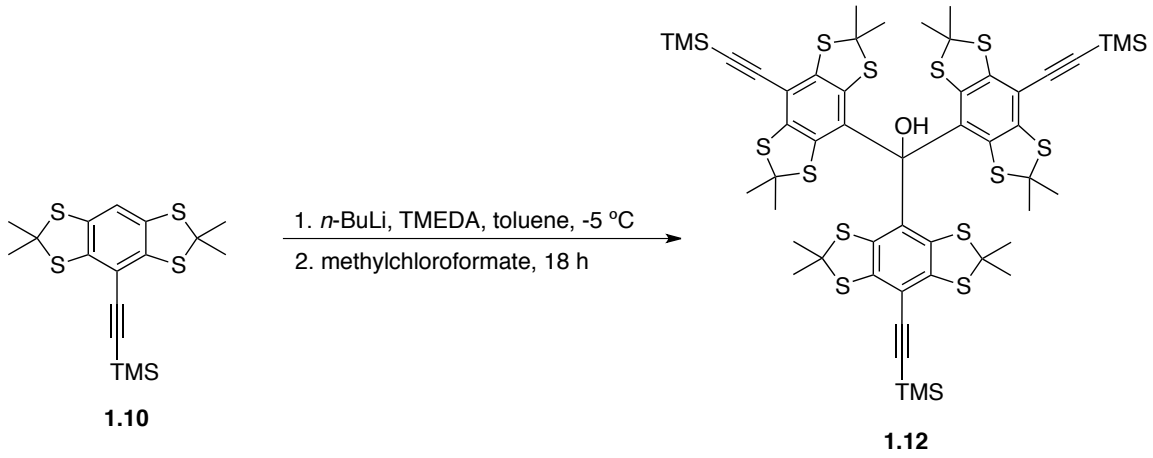
Experimental: Mono-substituted TMS trityl alcohol (**1.11**)



1.10 **1.11**

(2,2,6,6-tetramethyl-8-((trimethylsilyl)ethynyl)benzo[1,2-*d*:4,5-*d′*]bis([1,3]dithiole)-4-yl)bis(2,2,6,6-tetramethylbenzo[1,2-*d*:4,5-*d′*]bis([1,3]dithiole)-4-yl)methanol (1.11). A flame dried 100 mL RBF containing a magnetic stirbar and **1.10** (1.5381 g, 4.026 mmol, 3.0 eq) was purged with N_2 and charged with anhydrous toluene (20 mL). Freshly distilled TMEDA (0.607 mL, 4.026 mmol, 3.0 eq) was added and the contents were cooled to -78 ºC. *n*-BuLi (2.7 M in hexanes, 1.50 mL, 2.98 eq) was added dropwise and the reaction was allowed to stir for 2 h. Compound **1.7** (0.8025 g, 1.342 mmol, 1.0 eq) was dissolved in toluene (24 mL) and added via cannula upon which the reaction turned a dark olive green. The organic phase was extracted with DCM (3 x 10 mL), and washed with brine (20 mL). The organic phase was dried, filtered and evaporated under reduced pressure to yield a crude oil. The crude oil was purified with column chromatography (silica, 1% EtOAc in hexanes) to yield **1.11** as a yellow solid.

Experimental: Trimerization of **1.10** to yield **1.11**



1.10 **1.12**

Tris(2,2,6,6-tetramethyl-8-((trimethylsilyl)ethynyl)benzo[1,2-*d*:4,5-*d'*]bis([1,3]dithiole)-4-yl)methanol (1.12). A flame dried 25 mL RBF containing magnetic stirbar and compound **1.10** (500 mg, 1.308 mmol, 1.0 eq) was purged with N_2 and charged with anhydrous toluene. Freshly distilled TMEDA (0.1975 mL, 1.3089 mmol, 1.0 eq) was added and the reaction was cooled to -5 ºC. *n*-BuLi (2.0 M in hexanes, 0.7198 mL, 1.439 mmol, 1.1 eq) was added dropwise and the content turned a dark deep red color. The ice bath was removed after 30 min and the reaction was allowed to stir for 1.5 h. Methylchloroformate (0.033 mL, 0.4319 mmol, 0.33 eq) was added and the reaction was allowed to stir for 16 h. The organic phase was extracted with DCM (3 x 10 mL), and washed with NH_4Cl (20 mL) and brine (20 mL). The organic phase was dried, filtered and evaporated under reduced pressure to yield a crude oil. The crude oil was purified with column chromatography (silica, 1% EtOAc in hexanes) to yield **1.12** as a dark red oil (11 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.28 (s, 27H), 1.23 (s, 1H), 1.80 (s, 36H); ^{13}C NMR (100 mHz, $CDCl_3$): δ -0.05, 13.7, 22.0, 31.8, 64.7, 100.9, 104.6, 111.9, 115.9, 134.7, 139.5; HRMS=1174.1864; R_f = 0.61

CONCLUSION

In the future, we hope to continue to build the trityl radical library by attaching various R groups onto compound **1.4** and either trimerizing them to form symmetrical trityl radicals, or through addition to compound **1.7** to develop potential non-symmetric trityl radicals. The future will be focused on developing other trityl radicals. We will also begin to look at the linkage of these types of compounds to proteins in the appropriate linker strategy. We hope to be able to remove the TMS group to a terminal alkyne and then use the resulting alkyne in a click reaction as shown below.

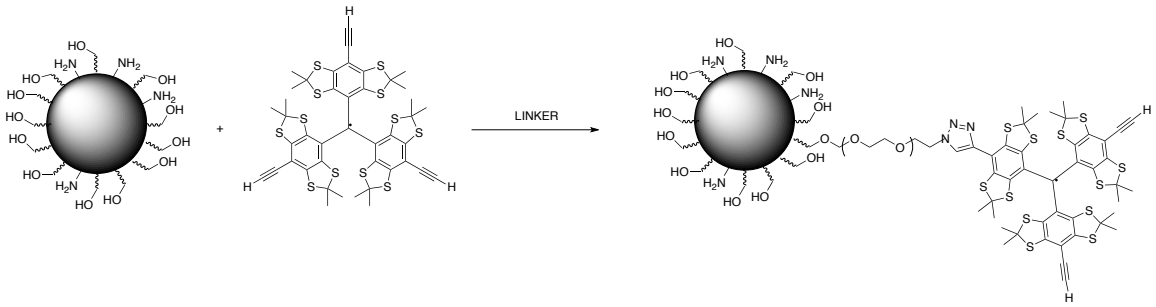


Figure 10: Click Reaction

With access to different trityl radicals, the ability for orthogonal protein tagging is made possible. The goal is to develop a trityl radical library to use as biological sensors via EPR that will have multiple applications. Applications include the testing of these EPR spin probes for conjugation to BuChE, treatment of enzymes inhibited by OP's in order to gain information of the bioscavenger, and apply this technology to other proteins to better understand their distribution *in vivo.* The development of these possible image-enhancing triarylmethal radicals will allow for effective spin labeling and a greater

understanding of molecular oxygen concentrations *in-vivo*.